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# SCIENCE

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## AVOGADRO'S LAW AND THE ABSORPTION OF WATER BY ANIMAL TISSUES IN CRYSTALLOID AND COLLOID SOLUTIONS<sup>1</sup>

### I. STATEMENT OF THE PROBLEM

FIFTEEN years ago, on an occasion similar to this, the writer stated that if the constitution of matter is the main problem of the physicist the constitution of living matter is the main problem of the biologist. To-day I will discuss the applicability of Avogadro's law, one of the most fundamental laws underlying the constitution of matter, to a group of life phenomena, namely the regulation of the amount of water in animal cells and tissues. According to Avogadro's law equal volumes of gases at the same temperature and pressure contain an equal number of molecules; or, in other words: In the gaseous state equal numbers of any kind of molecules enclosed in equal volumes have the same pressure at the same temperature. This law was extended to solutions by van't Hoff in the following form: All dissolved substances produce upon a membrane which prevents their diffusion but allows water to diffuse an osmotic pressure equal to that which would be produced by gaseous matter containing the same number of molecules in the same volume. Combining Avogadro's and van't Hoff's law we may state that the same number of molecules of any kind of matter produce at the same temperature and volume the same pressure upon the walls which prevent their diffusion.

<sup>1</sup> Read by title in the Botanical Section of the Cleveland meeting of the Society of American Naturalists, December, 1912.

It is well known that Pfeffer constructed cells of clay whose pores were filled with a precipitate of ferrocyanide of copper and that the walls of these cells allowed water, but not salts or sugars, to diffuse through. When such a cell with semi-permeable walls is filled with a salt or sugar solution and put into a larger vessel containing distilled water, the latter will diffuse into the cell and raise the level of the liquid until the hydrostatic pressure is equal to the osmotic pressure of the solute. Such a cell could therefore be used for the determination of the molecular weight of any dissolved substance which can not pass through its walls.

Now, if it be true that living cells are surrounded by semi-permeable walls and that the osmotic pressure regulates the exchange of water between the cells (or tissues) and the liquids of the animal body, it should also be possible to use living cells as osmometers for the determination of the molecular weight of sugars or salts.

The reader is aware that this possibility had already been demonstrated for plant cells (*Tradescantia*) by de Vries before van't Hoff had discovered the applicability of Avogadro's law to liquids and that de Vries's observations induced van't Hoff to interest himself in this problem. At the time of de Vries's work there was a difference of opinion concerning the molecular structure of the sugar raffinose and three different formulæ were offered, one giving the sugar the molecular weight 396, the second 594 and the third 1,188.

By determining the concentrations of cane sugar and raffinose which cause plasmolysis, *i. e.*, the shrinking of the protoplasm of the *Tradescantia* cells, de Vries found that a 3.42 per cent. cane sugar solution and a 5.96 per cent. raffinose solution had the same physiological effect. On the basis of the assumption that solutions with

an equal attraction for water have the same number of molecules in equal volumes, and since the molecular weight of cane sugar is 342, the molecular weight of raffinose should be  $(5.96/3.42) \cdot 342 = 596$ . In this way it was established by de Vries that the molecular weight of raffinose was 594 and the formula  $C_{18}H_{36}O_{16} + 5H_2O$  the correct one.<sup>2</sup> This formula for raffinose is to-day accepted by the chemists.

## II. EXAMPLES FOR THE APPLICABILITY OF AVOGADRO'S LAW TO THE OSMOTIC BEHAVIOR OF ANIMAL TISSUES

The oldest and, perhaps, best demonstration of the fact that the exchange of water between animal cells and the surrounding liquid is determined by Avogadro's law is furnished by Hedin's experiments on red blood corpuscles. His method consisted in the determination of the changes of volume of red blood cells in various solutions. The volume was determined with the centrifuge.

Hedin<sup>3</sup> first established the fact that the blood cells do not change their volume if put into solutions of the same molecular concentration as the blood, no matter what the nature of the solution, provided that the substance does not enter the cell.

### VOLUME OF BLOOD CORPUSCLES IN

.15m	KNO <sub>3</sub>	NaCl	NaCH <sub>3</sub> COO	CaCl <sub>2</sub>
	34.4	34.4	34.3	34.3

All the solutions had the same osmotic pressure as a .15m KNO<sub>3</sub> solution. The agreement of the results is such that the use of red blood corpuscles as osmometers for the determination of the molecular

<sup>2</sup> After Hoeber, *Physical. Chemie d. Zelle u. Gewebe*, 1911, p. 86; De Vries, *Jahrb. f. wissenschaft. Botanik*, Vol. 14, p. 427, 1884.

<sup>3</sup> Hedin, *Skand. Arch. f. Physiologie*, Vol. 5, pp. 207 and 238, 1895. (The tables quoted here are taken from Hamburger, "Osmotischer Druck und Ionenlehre.")

weight of dissolved substances would be warranted.

The following gives the results of the change in the volume of red blood corpuscles in isosmotic concentrations of  $\text{KNO}_3$  and  $\text{NaCl}$ .

Concentration	Volume of Blood Corpuscles in		Difference, Per Cent.
	$\text{KNO}_3$	$\text{NaCl}$	
.08m	48.6	50.2	-1.6
.1	46.3	48.2	-1.9
.12	43.2	44.2	-1.0
.13	42.5	43.4	-0.9
.14	41.4	42.2	-0.8
.15	40.2	41.0	-0.8
.16	39.9	40.4	+0.5
.17	39.7	39.6	+0.1
.18	39.4	39.2	+0.2
.20	39.1	38.0	+1.1
.22	39.2	37.3	+1.9
.24	38.7	36.8	+1.9
.26	38.3	36.5	+1.8
.30	37.2	36.8	+0.4

The agreement is best for .17  $\text{KNO}_3$  which was equi-molecular with the blood used in this case.

But even for concentrations differing from the molecular concentration of the blood the agreement is still surprisingly satisfactory, considering the fact that solutions which differ widely in their concentration from that of the blood corpuscles are liable to modify the permeability of the cells, as we shall see later; and that this injurious effect is influenced strongly by the chemical nature of the substance.

We may, therefore, state that equi-molecular solutions of salts cause practically the same change in the volume of red blood corpuscles.

The experiments on red blood corpuscles have the disadvantage that we can not well discriminate between the living and dead cell. In this respect the experiments on muscle are much more satisfactory. It had been known through the experiments of Nasse that in an m/8 solution of  $\text{NaCl}$  (and other salts of Na) the frog's muscle keeps

its weight. The writer showed sixteen years ago that (within certain limits of time) the same is true for solutions of  $\text{LiCl}$ ,  $\text{KCl}$ ,  $\text{MgCl}_2$ ,  $\text{CaCl}_2$ ,  $\text{SrCl}_2$  and  $\text{BaCl}_2$  equi-molecular with an m/8  $\text{NaCl}$  solution, while in solutions of lower concentration the muscle absorbs, in solutions of higher concentration it loses water. He concluded from this that the absorption of water by the muscle is determined by van't Hoff's law.<sup>4</sup> The number of molecules in solution, and not their chemical character, determines the exchange of water between muscle and surrounding solution.

I was interested to find out with what degree of accuracy Avogadro's law determines the exchange of water in the muscle. For this purpose a series of experiments were made with  $\text{NaCl}$  and various sugars in concentrations slightly below or above the point of isotony.

CHANGE IN WEIGHT OF A FROG'S MUSCLE WITHIN  
ONE HOUR IN PER CENT. OF ITS ORIGINAL  
WEIGHT IN

.1	.125	.150	.175m $\text{NaCl}$
+ 3.9%	0%	- 1.9%	- 1.9%

In a .1m solution of  $\text{NaCl}$  the muscle takes up water, in a .125m solution it keeps its weight, in a .15 solution it loses water. That the loss in a .175m solution was in this case not greater than in a .15 solution was accidental and probably due to the fact that different muscles vary somewhat in their osmotic pressure, owing to their previous history—*e. g.*, whether they had been more or less active. The influence of these inequalities can be eliminated by making a large number of experiments.

But, although the muscle is not quite as accurate an osmometer as the red blood corpuscles, the fact that its exchange of water is determined primarily by Avo-

<sup>4</sup>Loeb, *Pflüger's Archiv*, Vol. 69, pp. 14-20, 1897.

gadro's law is illustrated by the following experiment.

In an  $m/8$  solution of NaCl 85 per cent. of the molecules are dissociated into ions. If we want to prepare solutions of non-conductors (*e. g.*, sugars) of the same osmotic pressure as an  $m/8$  NaCl we must choose a concentration of .231m. At a concentration of a sugar solution of .200m a muscle must absorb water, while at a concentration of .250 and above it must lose water. We have made this experiment for three sugars, a mono-saccharide, grape sugar; a disaccharide, cane sugar; and a trisaccharide, raffinose. The following table gives the change in weight of the muscle in these solutions in one hour.

	.2m	.25m	.3m	.35m
Grape sugar.....	+2.9%	-1.6%	-4.1%	-7.7%
Cane sugar.....	+3.8%	-1.2%	-1.3%	-6.1%
Raffinose.....	+1.7%	-3.3%	-5.3%	-8.9%

The turning point between loss and gain of weight lies for all those sugars between the same limit of molecular concentration, namely, between .2 and .25m; and the most important fact is that the value for the three different sugars lies between the limits calculated on the assumption that the exchange of water between muscle and surrounding solution is determined by Avogadro-van't Hoff's law. As long as we are dealing in biology with only qualitative results there may always be some doubt in regard to the applicability of such a law to a life phenomenon, but if the results come out quantitatively identical with those calculated we may be pretty sure that the law holds good for these cases.

Miss Cooke<sup>5</sup> investigated in the writer's laboratory the gain of weight in the muscle in hypotonic and hypertonic NaCl solutions. Near the isotonic point the amount

of water increases at first slowly in almost a straight line with the dilution; but as the solutions become more dilute the amount of water taken up increases at a rate far greater than the rate of dilution of the solution. Miss Cooke points out that this may be due to some secondary change in the muscle caused by the dilute solution or the entrance of water into the muscle. In hypertonic solutions irregularities were noticeable, due probably to the varying condition of rest or activity in the individual muscle before the experiment.

The following gives a series of determinations in equi-molecular solutions of NaCl and cane sugar, by R. Webster.<sup>6</sup>

CHANGE IN WEIGHT OF FROG'S GASTROCNEMIUS  
AFTER ONE HOUR IN EQUI-MOLECULAR SOLUTIONS OF NaCl AND CANE SUGAR.

NaCl		Cane Sugar	
	Per Cent.		Per Cent.
m	-17	1.67m	-40
m/2	-13	.87m	-27
m/4	-7	.45m	-12
m/8	0	.23m	-1
m/16	+13	.12m	+11
m/32	+25	.05m	+26

While the gain in weight of the muscle in the equivalent hypotonic solution is practically the same in NaCl and cane sugar, in the hypertonic solutions of cane sugar the muscle loses more than in the equi-molecular solutions of NaCl. This is probably due to the fact that solutions of cane sugar have an abnormally high osmotic pressure and that this anomaly seems to be more pronounced the higher the concentration.

The number of animal organs in which the applicability of Avogadro's law can be tested is limited, since for this purpose it is necessary that the tissues have no pores or capillary spaces in which the surround-

<sup>5</sup> *Journal of Physiology*, Vol. 22, p. 137, 1898.

<sup>6</sup> R. Webster, University of Chicago Decennial Publications, Vol. X., p. 105, 1902.

ing solution can adhere. Tissues with a spongy structure can not be used for exact measurements. Siebeck<sup>7</sup> has recently been able to carry out a series of experiments on the kidneys of the frog. The measurements were not as satisfactory as in the case of the red blood corpuscles and the muscle, but sufficiently accurate to leave no doubt of the main result, which is as follows: In solutions of NaCl, NaBr, LiCl, and LiBr, KCl, CaCl<sub>2</sub>, MgCl<sub>2</sub> and cane sugar equi-molecular with a .7 per cent. Ringer solution the kidneys neither take up nor lose water. Siebeck points out that these solutions have no other quality in common except the same number of molecules, and that hence the osmotic pressure determines the exchange of water between the kidneys and the surrounding solution. Mathews's experiments indicate that the same is true for the living nerve.<sup>8</sup>

According to Meigs<sup>9</sup> smooth muscle preparations do not behave as if they were surrounded by semi-permeable membranes; but the smooth muscle of the stomach which he used in his experiments can not be obtained in as natural a condition as that in which striped muscle or blood corpuscles or the kidneys are available. It would be of interest to repeat these experiments on smooth muscle, which can be obtained with as little alteration of its natural surface, as, *e. g.*, striped muscle or kidneys or red blood corpuscles.

### III. ABSORPTION OF WATER BY COLLOIDAL SOLUTIONS

The natural media surrounding animal tissues are solutions which contain dis-

solved proteins in addition to certain salts. Some authors seem to take it for granted that the force by which such protein solutions or colloidal solutions in general absorb water is not determined by Avogadro's law, and some go so far as to state that the regulation of the distribution of water between cells and blood is solely determined by the proteins and not by the salts. Such ideas are contradicted by theory as well as by experiment. We can show by the use of living cells as osmometers that colloidal solutions behave exactly as Avogadro's and van't Hoff's laws demand.

Avogadro-van't Hoff's law demands that a grammolecular solution of any dissolved substance contain the same number of molecules—leaving aside temporarily the facts of dissociation—and this number  $N$  is Avogadro's constant. As is well known, the value of  $N$  has been determined for molecules with an astonishing degree of agreement by the most diverse methods. Thus Rutherford found  $N=62.10^{22}$  by counting the  $\alpha$  particles given off by a given quantity of radium per second; and Dewar as well as Boltwood obtained identical values,  $71.10^{22}$ , by a similar method.

A method of calculating  $N$  from the constants of heat radiation gave, according to a theory of Lorentz,  $N=62.10^{22}$ . Lord Rayleigh determined the number  $N$  of molecules in a grammolecule from the diffusion of light from the sky and found  $N$  about  $70.10^{22}$ .<sup>10</sup>

It would seem *a priori* perhaps doubtful whether, or not the suspended particles of a colloidal solution behave like molecules. Perrin undertook to solve this problem. He points out that van't Hoff's law (for properly dilute solutions) is applicable, no

<sup>7</sup> Siebeck, *Pflüger's Archiv*, Vol. 148, p. 443, 1912.

<sup>8</sup> A. P. Mathews, *Am. Jour. Physiol.*, Vol. II., p. 455, 1904.

<sup>9</sup> *Journal of Exper. Zoology*, Vol. 13, p. 498, 1912.

<sup>10</sup> These data are taken from Arrhenius, "Theories of Solutions," New Haven, 1912, pp. 25 and 26.

matter whether molecules are small or large, light or heavy.

Can we not suppose under these circumstances that there is no limit of size for the particles which obey this law? Should it not be possible that even visible particles obey this law exactly, so that a granule, agitated by brownian movement, counts neither more nor less than an ordinary molecule in regard to the action of its shocks on a wall which blocks its progress? Or, in other words, may we not assume that the laws of perfect gases can be applied to emulsions made of visible granules.<sup>11</sup>

It is well known that Perrin determined the value of  $N$  from the measurement of the relative distribution of the suspended particles of an emulsion of mastix in a vertical column and found  $N=62.2 \cdot 10^{22}$ . Almost identical values were obtained by him by three other methods. This proves that as far as osmotic pressure is concerned the suspended particles of an emulsion behave exactly like the molecules of a gas.

Direct osmometric measurements of the osmotic pressure of proteins gave very low figures, as was to be expected from their high molecular weight. R. S. Lillie found 30 to 40 mm. Hg for a 2 per cent. solution of albumen,<sup>12</sup> Starling for the proteins of blood serum 40 mm. Hg.

The question may now be asked whether or not the proteins in solution have other qualities by which they attract water than their osmotic pressure? This question can easily be answered by using living cells as osmometers. We have seen that an m/8 NaCl or a .231m sugar solution are isotonic with a frog's muscle (gastrocnemius). The latter solutions have an osmotic pressure of 3933 mm. Hg. We have also seen that as an osmometer a frog's gastrocnemius may be considered accurate at the point of isotony within .03m sugar. Hence the osmotic pressure

of the proteins of the blood is below the limit of sensitiveness of the muscle as an osmometer; or, in other words: if the attraction of water by blood is determined solely by the osmotic pressure of the blood that part of its total osmotic pressure which is furnished by the proteins must be negligible. This is indeed the case.

I asked my assistant, Dr. Beutner, to compare the change in weight of a frog's muscle in various solutions with and without proteins.<sup>13</sup>

CHANGE IN WEIGHT OF TWO GASTROCNEMII OF  
THE SAME FROG

	Muscle a in m/8 Ringer, Per Cent.	Muscle b in m/8 Ringer + 2 Per Cent. Gelatine. Per Cent.
After 1 hour . . . .	— 1.6	— 2.5
After 3 hours . . .	— 3.5	— 4.3
After 8 hours . . .	— 3.2	— 4.7
After 22 hours . . .	— 4.4	— 3.8
After 29 hours . . .	— 4.2	— 3.8

The differences in the two rows of figures are slight in comparison with the experimental errors and show that the addition of gelatine does not influence noticeably the absorption of water.

In a second experiment it was ascertained that in blood serum which was freed from the greater part of its salts by dialyzing it for 40 hours through parchment paper the muscle gains exactly as much in weight as it does in a NaCl solution of the same osmotic pressure. The serum had after dialyzation a freezing point depression of .03°, corresponding to a concentration of NaCl of about m/120.

CHANGE IN WEIGHT OF TWO GASTROCNEMII OF  
THE SAME FROG

	Muscle a in Dialyzed Serum, Per Cent.	Muscle b in m/120 Ringer, Per Cent.
After ½ hour . . .	+ 22.3	+ 24.7
After 1 hour . . .	+ 34.0	+ 38.0
After 2½ hours .	+ 51.0	+ 53.6

<sup>11</sup> Perrin, "Les Atomes," Paris, 1912, p. 128 ff.

<sup>12</sup> Lillie, *Am. Jour. of Physiol.*, Vol. 20, p. 197.

<sup>13</sup> R. Beutner, *Biochemische Zeitschr.*, Vol. 48, p. 217, 1913.

These two series of results may be considered as identical within the limits of error. They prove that proteins in solution attract water from the muscle solely according to their osmotic pressure and not according to any other real or assumed quality. They prove also that the salts and not the proteins of the blood determine the exchange of water with the tissues.

R. Lillie has shown that the addition of acid increases the osmotic pressure of a protein solution, but the increase is too small, even in dialyzed serum, to influence the velocity of water absorption, as the following figures show.

CHANGE IN WEIGHT OF TWO MUSCLES

	Muscle <i>a</i> in 50 c.c. Dialyzed Serum +1 c.c. N/10 HCl, Per Cent.	Muscle <i>b</i> in Dialyzed Serum without Acid, Per Cent.
After 1 hour ...	+ 33.0	+ 35.0
After 2 hours ..	+ 43.0	+ 46.0
After 4 hours ..	+ 52.0	+ 49.0
After 6 hours ..	+ 45.0	+ 43.0

I think all these experiments prove conclusively, (1) that colloidal solutions influence the absorption of water in the tissues solely by their osmotic pressure; and (2) that this osmotic pressure is so small in comparison with that of the salts in the liquids of the body that their influence is in general practically negligible.<sup>14</sup>

#### IV. PERMEABLE AND NON-PERMEABLE SOLUTES

The existence of semi-permeable walls which allow the water but not the solute

<sup>14</sup> The erroneous idea that the attraction of water by blood serum is determined by proteins may have originated through the arbitrary assumption that the phenomenon of imbibition found in solid colloids is retained by the colloids in solution. This reasoning is, however, not warranted, since it is possible that in solid colloids conditions for the absorption of water may exist which cease to exist when the colloid is dissolved.

to diffuse is the prerequisite for the manifestation of osmotic pressure. It is well known that Overton showed that dissolved substances which diffuse easily into the cell do not produce any osmotic effect. The permeability of cell walls for alkaloids can be directly demonstrated by their reaction with the tannic acid of the cells, and Overton showed that when added to an isotonic salt solution the alkaloids will not alter the water equilibrium. The substances which thus diffuse readily into the cells are alcohols, aldehydes and many other compounds which often show a relatively high degree of solubility in lipoids. The difference in the behavior of substances which are soluble in the cell wall (like alcohol) and non-soluble (like sugar) is illustrated by the following experiments by Overton.<sup>15</sup>

In 0.2 per cent. NaCl + 2.66 per cent. mannit (together equi-molecular with .7 per cent. NaCl) the gastrocnemius of a frog kept its weight for nine hours unaltered. In 0.2 per cent. NaCl + 2 per cent. methyl alcohol (approximately equi-molecular with a 1.6 per cent. NaCl solution), the muscle absorbed water as powerfully as in a pure 0.2 solution of NaCl. Only those substances which can not diffuse into the cell can assist in the exchange of water between tissues and the liquids of the body; exactly as Avogadro's law demands.

It is generally assumed that there is only one type of semi-permeable membranes, namely, those permeable for water, but not for neutral salts or sugars. This type is the most common. But there are at least two other types of semi-permeability. Some membranes are permeable for water as well as salts and sugars, but not for colloids. The fertilization membrane of the sea urchin egg (*Strongylocentrotus*

<sup>15</sup> Overton, *Pflüger's Archiv*, Vol. 92, p. 115, 1902.



*purpuratus*) belongs to this type.<sup>16</sup> On the other hand, there are membranes which are neither permeable for salts nor for water. This type of membranes is represented by the eggs of *Fundulus*, and apparently *Fundulus* itself, and probably by a large number of other aquatic vertebrates.

All these membranes are, however, permeable for gases ( $O_2$  and  $CO_2$ ) dissolved in water.

V. THE APPARENT CONTRADICTION BETWEEN  
THE PHYSIOLOGICAL EFFICIENCY OF  
SALTS AND THE SEMI-PERME-  
ABILITY OF CELL WALLS

We will now discuss a difficulty which the idea of impermeability of the cells for salts has to meet. In a pure NaCl solution the frog's muscle begins to twitch, while the addition of a little Ca inhibits this effect. It is difficult to assume that salts act without diffusing into the cell, and yet the phenomena of isotony are incomprehensible without the assumption of practical impermeability of the cell for salts and sugars. This difficulty is apparently relieved by the investigations of Loeb and Beutner<sup>17</sup> on the potential differences at the limit between living organs and the surrounding solution of electrolytes. If we form a cell of the type: solution of electrolyte (concentrated); living organ; solution of electrolyte (dilute), the dilute solution is positive towards the concentrated; and the more so the greater the dilution. This seems to be a fact for most living tissues. From this it follows that living tissues are reversible for kations. We were, moreover, able to show that the electromotive forces of such cells obey

Nernst's law, according to which the E.M.F. varies with the logarithm of the concentration. But the most surprising fact we found was that the living cells are reversible in regard to practically any kation, while the potential differences heretofore observed by physicists at the limit of two phases were only reversible for one kation. These results are only intelligible on one of two assumptions: either the surface of all living organs contains already traces of all metals, which is not very probable; or traces of the electrolytes react with the surface of the living organ. If the latter is true we can understand that electrolytes can influence the life phenomena and yet produce the theoretically calculated osmotic pressure upon the walls of living organs.

The writer suggested thirteen years ago that chemical reactions occur between the electrolytes and certain parts of the "protoplasm"—proteins or fatty compounds—which result in the formation of ion or metal-proteins or soap-like compounds, and that these reactions explain the influence of the electrolytes upon muscular twitchings, heart beat, etc. It is well known that the action of salts like NaCl or  $CaCl_2$  or KCl upon the heart beat or muscular twitchings is—within certain limits of concentration and time—perfectly reversible, and this reversibility is characteristic of this action. Such a complete reversibility of chemical reactions is intelligible if only traces of the salts enter into reaction with the substances contained in the living organ.

Beutner reached the conclusion on the basis of thermodynamical calculations that the potential differences observed by us at the junction of living organs and surrounding solution demand the assumption of a chemical reaction between traces of

<sup>16</sup> Loeb, *Roux's Archiv*, Vol. 26, p. 82, 1908.

<sup>17</sup> Loeb and Beutner, *Biochemische Zeitschr.*, Vol. 41, p. 1, 1912; Vol. 44, p. 303, 1912.

the electrolyte and the surface of the living organ.<sup>18</sup>

#### VI. THE ALTERATION OF THE SEMI-PERMEABILITY OF CELL WALLS BY ELECTROLYTES

Those who have worked with Pfeffer's semi-permeable cells report that it is very difficult to maintain cells in the state of ideal semi-permeability. It seems that the semi-permeability of living organs suffers also if they are exposed to solutions abnormal in concentration or constitution or both. The normal medium for the cells of our body is a liquid which contains NaCl, KCl and CaCl<sub>2</sub> in a proportion which is almost identical with the one in which those salts appear in the ocean. In such solutions, if their concentration is right, the cell walls may keep their specific semi-permeability as long as they live. If, however, the cells or organs are put into solutions of other salts, or of solutions with only one of the three salts just mentioned, the semi-permeability is lost sooner or later and death follows. The possible explanation is that the interactions between salts and surface of the living organ lead in this case to a modification of the physical qualities of the surface of the living organ.

In 1899 the writer found that the newly fertilized eggs of a marine fish, *Fundulus*, will develop normally in distilled water and the fish will hatch. If, however, the eggs are put into an  $m/2$ , or still better  $\frac{5}{8}m$ , NaCl solution immediately after fertilization they will die without forming an embryo. If a small but definite amount of a salt with a bivalent metal is added to the NaCl solution the eggs will form embryos. The explanation of these facts is as follows:

If the newly fertilized eggs are put into a  $\frac{5}{8}m$  NaCl solution the NaCl makes the egg membranes, which are naturally impreg-

nable to salts, gradually permeable. NaCl enters the egg and the germ is killed. If a little Ca is added to the NaCl this modification of the membrane is inhibited, no NaCl enters the egg and the germ can live and develop. In distilled water the membrane remains also impermeable to salt (and to water) and the embryo likewise develops.<sup>19</sup>

The writer has recently published a series of experiments which furnish a proof for this assumption.<sup>20</sup> Only the general character of this proof can be indicated here. The eggs of *Fundulus* have a specific gravity of a little over 1.0580 (corresponding to  $\frac{11}{8}m$  NaCl solution). If such eggs several days after fertilization are put into a 3m solution of NaCl + KCl + CaCl<sub>2</sub> in the proportion in which these three salts occur in the sea-water, the eggs will float at the surface of the solution for four days or longer. Then they will shrink and fall to the bottom of the test tube. The membrane is impermeable to water and salts and remains so for a series of days even in a 3m solution of NaCl + KCl + CaCl<sub>2</sub>. Finally it becomes permeable for water and to a lesser degree for salts and the eggs shrink and fall to the bottom. If, however, the eggs are put into a 3m solution of NaCl they will also float at first; but the increase in permeability and the shrinking begins in three hours. If we put the eggs into a solution of CaCl<sub>2</sub> from  $\frac{10}{8}m$  to  $\frac{21}{8}m$  they float at first, but the shrinking begins in 20 minutes and the eggs sink to the bottom. If we put the eggs into a mixture of 48 c.c. 3m NaCl + 2 c.c.  $\frac{10}{8}m$  CaCl<sub>2</sub> they will

<sup>19</sup> Loeb, SCIENCE, Vol. 34, p. 653, 1911. Reprinted in "Mechanistic Conception of Life," Chicago, 1912; *Pflüger's Archiv*, Vol. 107, p. 252, 1905.

<sup>20</sup> Loeb, *Biochemische Zeitschr.*, Vol. 47, p. 127, 1912.

<sup>18</sup> Beutner, *Biochem. Zeitschr.*, Vol. 47, p. 73, 1912.

float for three days, before they begin to shrink and fall to the bottom. This and similar experiments show that the specific semi-permeability of an animal membrane is influenced by the nature of the solutions to which it is exposed. In physiologically balanced solutions, *e. g.*,  $\text{NaCl} + \text{KCl} + \text{CaCl}_2$  in the proportion in which these salts exist in the sea-water, this specific semi-permeability is preserved longest, if not permanently. In solutions of one salt only the permeability is as a rule increased as soon as the concentration of the salt in the solution exceeds a certain limit. This limit varies for different salts and varies also for the same salt in different animals. This is the reason why a pure  $\text{NaCl}$  solution becomes injurious more rapidly to one form of organism than to another. The reader will notice that in this field of phenomena the chemical nature of the solution is of primary importance.

This dependence of the preservation of semi-permeability upon the chemical nature of the surrounding solution suggests the possibility of a chemical interaction between cell walls and surrounding solution in which the chemical and physical properties of the semi-permeable wall are modified.

#### VII. THE DIFFERENCE IN THE MODE OF ABSORPTION OF WATER IN LIVING AND DEAD CELLS

We may conclude from the preceding data that the salts of the surrounding solution react chemically with the colloids of the surface of the cell and that the products thereby formed determine the degree of permeability or impermeability of the cell walls.

These changes vary with the chemical nature of the salt. In some solutions the permeability is preserved much longer than in others. Thus the muscle keeps its

normal function much longer in a  $\text{NaCl}$  or a  $\text{LiCl}$  solution than in a solution of  $\text{KCl}$  or  $\text{CaCl}_2$ . As long as the muscle is irritable or even some time after it ceases to be irritable it obeys Avogadro's law in the solutions of pure salts. After 18 hours, however, the muscle is killed in pure isotonic solutions of  $\text{KCl}$  as well as of  $\text{CaCl}_2$ , while it is still alive in solutions of  $\text{NaCl}$  and  $\text{LiCl}$ . At that time it continues to obey the law of Avogadro in  $\text{NaCl}$  and  $\text{LiCl}$ , but no longer in  $\text{KCl}$  and  $\text{CaCl}_2$ .

CHANGE IN WEIGHT OF A GASTROCNEMIUS OF A FROG AFTER 18 HOURS IN SOLUTIONS EQUI-MOLECULAR WITH  $m/8 \text{ NaCl}$  <sup>21</sup>

	Per Cent.		Per Cent.		Per Cent.		Per Cent.
$\text{LiCl}...$	-1	$\text{NaCl}..$	+ 6	$\text{KCl}..$	+45.7	$\text{CaCl}_2...$	-20
$\text{LiBr}...$	-1	$\text{NaBr}..$	+ 7	$\text{KBr}...$	+41		
$\text{LiJ}....$	-3	$\text{NaJ}...$	+10	$\text{KJ}....$	+45		

While the muscle undergoes no, or only slight, changes in weight in the isotonic solutions of  $\text{Na}$  and  $\text{Li}$  salts it absorbs a considerable amount of water in  $\text{KCl}$ , loses water in  $\text{CaCl}_2$ .

Overton, who later repeated and confirmed these results, explained them on the assumption that the normal muscle is permeable for  $\text{KCl}$ , but not for the other salts. In a pure  $\text{KCl}$  solution the salt gradually enters the muscle, whereby the osmotic pressure of the muscle is increased and the latter swells; while in a  $\text{CaCl}_2$  solution the  $\text{KCl}$  naturally contained in the muscle leaves the muscle and the diminution of the number of molecules in the muscle makes the surrounding originally isotonic solution hypertonic for the muscle. One fails to see, on such an assumption, why the muscle does not also lose water in a  $\text{NaCl}$  solution. It has been demonstrated

<sup>21</sup> Loeb, *Pflüger's Archiv*, Vol. 75, p. 304, 1899; "Studies in General Physiology," Vol. II., p. 511, Chicago, 1906.

that the normal gastrocnemius of a frog contains little or no NaCl, but comparatively much KCl, while in the blood the reverse is the case. From this it has been plausibly argued that the NaCl of the blood can no more enter the muscle than can  $\text{CaCl}_2$ . One fails, however, to see how on this assumption the fact can be explained that in a pure NaCl or LiCl solution the muscle keeps its weight or gains slightly, while in  $\text{CaCl}_2$  it loses considerably in weight, since in a pure NaCl solution the K-salts should leave the muscle as well as in a  $\text{CaCl}_2$  solution.

In all hypertonic solutions the muscle loses water and the higher the concentration of the solution is the more water it loses. This is what should be expected according to Avogadro's law. But the writer noticed that if the muscles remain in the hypertonic solution they begin after a certain time to absorb water and the higher the concentration the sooner, so that finally the muscle begins to swell even in a grammolecular solution of NaCl!

This paradoxical behavior finds its explanation in the writer's observations on *Fundulus* eggs, which show that the impermeability of the membrane for salts and sugars is destroyed the more rapidly the higher the concentration of a solution. As soon as this impermeability is destroyed the cells no longer follow Avogadro's law, but show a behavior which is comparable to that of a piece of solid gelatine, i. e., water is absorbed not by osmotic forces but by "imbibition." The degree of imbibition varies with the nature of the surrounding salt and on this assumption the writer explained the differences of behavior of dead muscle in KCl and  $\text{CaCl}_2$  solutions.<sup>22</sup>

<sup>22</sup> Loeb, *l. c.*

#### VIII. THE ANTAGONISM BETWEEN ACIDS AND SALTS

The influence of the chemical character of the surrounding solutions upon imbibition in the dead muscle is well illustrated by the following observations:

The writer showed sixteen years ago that the muscle absorbs water from an m/8 solution of NaCl if HCl (or any other acid) is added.<sup>23</sup> In this case the law of Avogadro-van't Hoff is apparently violated. It was pointed out by Overton that in this case the acid destroys the semi-permeability of the muscle and that the subsequent absorption of water was a mere phenomenon of imbibition. It had been shown previously that the swelling of a solid plate of gelatine in water is increased if acid is added.

In a subsequent paper it was shown by the writer that there exists a curious antagonism between salts and acids. While a muscle swells in an m/8 NaCl solution if the latter is rendered acid, it does not swell, or it may be even dehydrated in an acid solution when the concentration of the NaCl in the solution is sufficiently raised; notwithstanding the fact that in the highly concentrated *neutral* NaCl solution the muscle will finally absorb water.

The following table illustrates this:

Neutral NaCl		m/110 HCl in NaCl	
Concentration of NaCl, Per Cent.	Increase in Weight in 18 Hours, Per Cent.	Concentration of NaCl, Per Cent.	Increase in Weight, Per Cent.
4.9	+6	4.9	-36
1.22	-2	1.22	+22.2
0.7	+7	0.7	+40

While the muscle gains in weight in eighteen hours in a neutral 4.9 per cent.

<sup>23</sup> Loeb, *Pflüger's Archiv*, Vol. 69, p. 1, 1897; Vol. 71, p. 457, 1898. "Studies in General Physiology," Vol. II, pp. 450 and 501, Chicago, 1906.

NaCl solution, it loses considerably in an m/110 acid 4.9 per cent. NaCl solution.<sup>24</sup>

The writer also pointed out that the chemical character of the salt was of great influence in this phenomenon. While it required a high concentration of NaCl and of acid to cause a dehydration, the same was accomplished by a much weaker solution of  $K_2SO_4$ . In a neutral isotonic solution of  $K_2SO_4$  the muscle lost 4 per cent. in eighteen hours, but when 10 c.c. m/10 HCl were added to 100 c.c. isotonic  $K_2SO_4$  solution the muscle lost 22 per cent. of its weight! The concentration of  $K_2SO_4$  required to inhibit the swelling effect of the acid is therefore much smaller than that of NaCl.<sup>25</sup>

When a muscle is put into a .04 solution of  $Na_2SO_4$  which is rendered m/200 acid through the addition of  $H_2SO_4$  the muscle first absorbs water and later is dehydrated, as the following experiment by Beutner<sup>26</sup> shows:

CHANGE IN WEIGHT OF MUSCLE IN .04m  
 $Na_2SO_4$  — N/200  $H_2SO_4$

1 hour .....	+ 9.9
3 hours .....	+ 12.3
6 hours .....	+ 1.8
24 hours .....	— 19.3
32 hours .....	— 25.2
96 hours .....	— 31.2

The interpretation of this result offers no difficulty. As Beutner pointed out, the muscle obeys the law of van't Hoff, as long as it is alive, or more correctly, as long as the semi-permeability of its membrane is preserved. Since a .04m solution of

$Na_2SO_4$  is hypotonic, the muscle must absorb water in such a solution. As soon as the semi-permeability of the muscle is lost and the acid diffuses into the muscle it behaves altogether differently, namely, like a piece of gelatine. Proctor has shown that solid gelatine swells in a salt solution as well as in an acid solution, while it is dehydrated in a combination of both; and the same is true for the dead muscle.

#### IX. INTERNAL CHANGES IN THE MUSCLE

Miss Cooke showed that the fatigued muscle of a frog is no longer isotonic with an m/8 solution of NaCl, but with a 3m/16 solution. The fatigue has, therefore increased the osmotic pressure of the muscle.<sup>27</sup> The writer suggested that in this case lactic acid was formed and that this lactic acid was the cause of the absorption of water by the muscle in an isotonic solution.<sup>28</sup> The lactic acid might act as a catalyzer in certain reactions, whereby the osmotic pressure would be raised in the muscle. Others assume that some colloid in the muscle is caused to swell by the acid. This could only refer to some solid colloid. But it is difficult to understand how this could force the muscle to absorb more water. Proctor<sup>29</sup> has shown that solid colloids absorb not only *water*, but also *salts* (even in the presence of acid); if this be the case the swelling of solid colloids inside the muscle can only increase the osmotic pressure of the liquids contained inside the muscle cells, if relatively more water is absorbed than salt. Since no quantitative data are available on this point, it is useless to argue the question.

<sup>27</sup> In order to avoid misunderstandings it should be said that the fatigued muscle obeys Avogadro's law, only its point of isotony is increased.

<sup>28</sup> Loeb, *Pflüger's Archiv*, Vol. 71, p. 466, 1898.

<sup>29</sup> Proctor, "Kolloidchemische Beihefte," Vol. II., p. 262, 1911.

<sup>24</sup> Loeb, *Pflüger's Archiv*, Vol. 75, p. 302, 1899. Reprinted in "Studies in General Physiology," Vol. II., p. 516, 1906.

<sup>25</sup> Loeb, *Pflüger's Archiv*, Vol. 75, p. 303, 1899; "Studies in General Physiology," Vol. II., p. 514.

<sup>26</sup> Beutner, *Biochem. Zeitschr*, Vol. 39, p. 280, 1912.

## CONCLUSION

I believe the facts mentioned in this address show that those animal cells or organs which lend themselves to exact measurements of osmotic changes obey the law of Avogadro-van't Hoff, as long as they are normal or alive: *i. e.*, they neither absorb nor lose water if put into solutions of any kinds of sugar or neutral salts which are equi-molecular with the blood; that they absorb water in solutions of lower osmotic pressure, lose water in solutions of higher osmotic pressure. If the law of Avogadro and van't Hoff is correct (which nobody doubts), this behavior of the tissues is the expression of the applicability of this law to the exchange of water between tissues and liquids of the body.

It does not often happen in biology that we are able to reduce life phenomena to a fundamental physico-chemical law to such an extent that we can not only predict the results qualitatively, but also quantitatively, as is the case in the application of Avogadro's law to the exchange of water between tissues and the liquids of the body.

If anybody wishes to supplant the law of Avogadro he must be able to offer a theory which allows a still closer approximation between calculated and observed results than is the case in the experiments on the absorption of water by animal cells or tissues. No such theory has thus far been offered.

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THE SPREAD OF THE BROWN-TAIL AND  
GYPSY MOTH

For several years Massachusetts has been spending large sums of money in fighting the brown-tail and gipsy moths. These insects have spread westward in their devastating course and are now recorded from a point

not far distant from the eastern boundary of New York. In February a conference was called in Boston by the state forester of Massachusetts for the purpose of bringing together not only those actually engaged in the fight with the gipsy and brown-tail moths, but those who are sure to be concerned in the near future. The New York State College of Forestry at Syracuse University is investigating the work of these insects and Dr. M. W. Blackman, forest entomologist of the college, was its representative in the conference in Boston and is ready to take up the fight against these insects when they appear in this state.

Interesting facts were brought out at this conference as to the spread of the gipsy moth. It is very certain that New York and other states about Massachusetts will soon be reached by these destructive insects and that unless more effective means are taken they will destroy not only forest trees but shade and ornamental trees over large sections of the state. As shade tree pests these insects can be controlled by spraying and destroying egg clusters, but these methods are used only at considerable expense and must be continued indefinitely. It seems probable that as soon as the parasites and diseases introduced from abroad which work upon and destroy these insects have become fairly established that they will aid man greatly against future serious outbreaks.

The gipsy moth problem of the future in the state of New York is a forestry problem, as the insect can not be fought in the forest by spraying but must be controlled and eventually eliminated by proper methods of forest management. Certain trees, such as the oak, willow and birch, are apparently more favorable and often seem necessary for the development of the caterpillars of the moths. Methods of forest management can be used which will remove these trees from the forest and thus destroy the most favored food of the pests. With these methods of proper forest management must go strict quarantine against lumber, cordwood and nursery products shipped in from infested areas. Some effort